Egypt. J. Bot. Vol. 47, pp. 99-118 (2007)

Genetically Construct *Saccharomyces cerevisiae* Strains Harboing α-Amylase Gene From Amylolytic Yeasts

A. M. El-Adl[1], K. A. Zaied[1], S. A. Ibrahim[2] and A. H. M. El-Zanaty[3]

[1]Department of Genetics, Faculty of Agriculture, Mansoura University, [2]Department of Genetics, Faculty of Agriculture, Ain Shams University and [3]Department of Microbial Biotechnology, Genetic Engineering and Biotechnology Research Institute, Minufiya University, Cairo, Egypt.

A HIGHLY active α-amylase gene secreted by five raw starch-degrading yeasts has been transferred to four *S. cerevisiae* strains using $CaCl_2$ and spheroplast transformation (PEG methods) . Lower percentage of transformation was obtained in response to direct transformation using $CaCl_2$, while transformation using PEG method showed higher percentages. In addition, PEG method yielded the maximum transformation percentage 4.0 %, whereas, that in $CaCl_2$ method was reached to 2.7 %. The higher number of transformation experiments using $CaCl_2$ was more than that produced by PEG which may due to lower regeneration of yeast spheroplasts on spheroplast transformation plates. Transformants were differed in their ability to grow on soluble starch due to the genetic background of various isolates contributing to the differential levels of α-amylase secretion which infuence their growth rate and genetic stability of expression α-amylase gene. All transformants were able to secrete extracellular α-amylase and expression of *α-Amy* gene was differed significantly in their activity to utilize soluble starch. Furthermore, transformants containing the *AMY* gene showed significant differences in halo size, which may be due to the different levels of expression of α-amylase gene. Recombinant isolates exerted significant differences in hydrolysis percentage of raw potato starch. Positive relation was achieved between ethanol production from raw potato starch and glucose values and the expression of α-amylase genes. Also, differences were obtained between amylolytic yeast strains and their transformants based on molecular weight of protein bands, the similarity degree and genetic distance.

Keywords : α-Amylase gene, Ethanol yielding, Genetic distance, Protein bands, Starch degradation, Transformation.

The yeast *Saccharomyces cerevisiae* has been used extensively for the production of many heterologous proteins, since it is safe eukaryotic microorganism with well established fermentation technology for large-scale production (Romanos *et al.*, 1992). Large amounts of yeast cells can easily be grown at lower cost than any other eukaryotic expression system. In addition, as the yeast *Saccharomyces cerevisiae* can not produce the starch degrading